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BIOLOGICAL EFFECTS OF FUEL AND EXHAUST COMPONENTS FROM SPACECRAFT DESCENT ENGINES EMPLOYING HYDRAZINE

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BIOLOGICAL EFFECTS OF FUEL AND EXHAUST COMPONENTS FROM SPACECRAFT DESCENT ENGINES EMPLOYING HYDRAZINE[1]

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SUMMARY

One of the objectives of the Viking mission is to determine the existence of extraterrestrial life on the Martian surface. A retrorocket engine powered by a catalytic decomposition of hydrazine fuel will be employed to soft-land the scientific instrumentation on the surface of Mars. It was imperative, therefore, to determine if hydrazine and its breakdown products in the engine exhaust would affect organisms at the sampling site. Experiments were done exposing microorganisms, in pure culture and in soils, to the rocket engine exhaust, the liquid hydrazine fuel, and some of the individual decomposition products of the latter. The results did not demonstrate that the gaseous exhaust products were hazardous to the microorganisms tested, but they did indicate that liquid hydrazine was lethal.

INTRODUCTION

In the final approach of the Viking lander on Mars, the retrorocket engine to be used is powered by a catalytic decomposition of hydrazine fuel to gaseous products by a stoichiometrically undefined process of the type:

$$(2a+b)N_2H_4 \rightarrow 2aNH_3 + (a+2b)H_2 + (a+b)N_2$$

It was estimated that at the landing site NH_3, the major product, might reach fleeting concentrations of approximately 30 percent of the Martian CO_2 atmosphere. In such an atmosphere the reaction also generates aniline, hydrocyanic acid, and in all probability carbazic acid ($H_2NNHCOOH$) and ammonium carbamate (NH_4OOCNH_2).[2] Further work by Martin-Marietta Corporation and others confirmed that the products depended on the grade of fuel used and the nature of the atmospheric environment. Purification of the "mil-spec" (ref. 1) hydrazine fuel lowered the yields of the decomposition side products, especially HCN. Nevertheless, it appeared that HCN at levels of 200–500 ppm might be encountered in the atmosphere at, and immediately surrounding, the Martian landing site. The possibility also exists that hydrazine fuel could contaminate the landing site by lander tank leakage, by unreacted N_2H_2 in the engine exhausts, or by the impact of the deorbit propulsion tanks with the aeroshell.

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[1]This work was supported by the Langley Research Center, National Aeronautics and Space Administration, Hampton, Virginia, which is responsible for the overall management of the Viking Project.

[2]Personal communication, Dr. Paul Fennessey, Martin-Marietta Company, Denver Division.

To evaluate the effects of the Viking descent engine products on organisms at the Martian landing site, the following studies were done in a CO_2 environment: sorption–desorption of HCN gas in soil; effect of HCN and NH_3 on the anaerobic respiration and survival of microorganisms in simulated Martian soil and in terrestrial soils; effect of liquid hydrazine fuel on the anaerobic respiration and survival of soil microorganisms; effect of rocket engine exhaust residues collected on glass rods on cultures of microorganisms; and effect of rocket engine exhaust on the anaerobic respiration of the microorganisms within simulated Martian soil.

MATERIALS AND METHODS

Sorption–Desorption of HCN Gas in Soil

Hydrogen cyanide was selected for study as the most reactive (but by itself, stable) of the side products of the decomposition of hydrazine fuel. The sorption experiments were conducted using soil in glass columns (1-cm I.D. × 10 cm) or thin layers of soil in glass petri dishes (1.5 cm × 15 cm) in a vacuum desiccator. The 100–120 mesh fractions of air-dried California soils were used. The soil columns were prepared by stacking weighed portions of soil between bronze screen dividers. This technique allowed individual segments to be removed for analysis. The stacked columns could be pumped down from either top or bottom, or from both simultaneously.

The apparatus used to expose thin layers of soils to gas was made from a 10-liter desiccator. Only twelve 1-g soil samples in small petri dishes were placed in each desiccator to ensure a generous access of the gases to the soils. During gas exposures the chambers were closed off from the vacuum system. At termination, the chambers were pumped clear and the samples held under dry nitrogen until assayed. No measurable leaks were detected in the apparatus.

A stainless steel–Pyrex glass manifold (fig. 1) was used to expose soils to simulated rocket exhaust products. The manifold design allowed various attachments to be made. Gas was metered with a differential gage calibrated from 2400 to 40 torr. Pressures less than 40 torr were obtained by measuring in the calibrated region, followed by successive expansions into previously calibrated volumes of the manifold system.

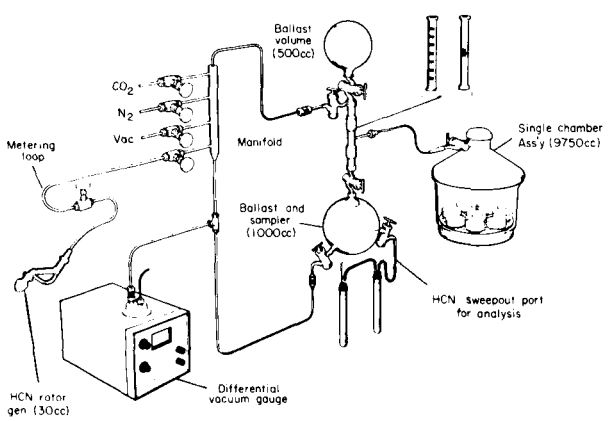

Figure 1.– Gas handling system for CO_2-HCN-NH_3

Outgassing of the soil samples was the limiting factor in the pumpdown rate of the vacuum system; the vacuum system was rated 135 liters per minute throughout. A dynamic pressure of 0.1 torr (0.15 torr static) was chosen for the starting condition in the experiments. After a soil had been outgassed once to this point and returned to higher pressures, any subsequent pumpdown proceeded at a faster rate.

Hydrogen cyanide gas was generated from reagent grade KCN and concentrated sulfuric acid. The gas was released from a rotor-generator attached to the vacuum manifold (fig. 1). The dry HCN gas was held in a separate bulb in the line. Hydrogen cyanide was diluted with CO_2 by a sequence expansion technique into spherical reservoirs of known volume. The gas manifold had the accessories needed for storage and handling of all gases. Mixing of the gases was ensured by using spherical reservoirs and allowing sufficient time for diffusion before permitting the mixture to enter the test chambers. In the diffusion studies of HCN, the soil columns and desiccators were first evacuated to a pressure of 0.1 torr, then brought to 10 torr CO_2 and equilibrated before admission of 10 torr CO_2 containing HCN.

The concentration of HCN in the CO_2-HCN mixture was verified by bubbling aliquots of the gas mixture through a two-stage scrubber containing 0.1N NaOH and analyzing this solution by a method based on the colorimetry of complexed cyanogen chloride (refs. 2–4). The same technique was used for analyzing sorbed HCN in soils. The soil columns were dismantled and one segment at a time was quickly poured into Erlenmeyer flasks containing 0.6N NaOH. The soil segments were extracted at room temperature for 16 hours and filtered to remove the soil particles (fig. 2). The same procedure was used with soils treated in desiccators. Blanks and standards for the colorimeter readings were obtained by extracting untreated samples of the particular soil and adding KCN to make a series of standards.

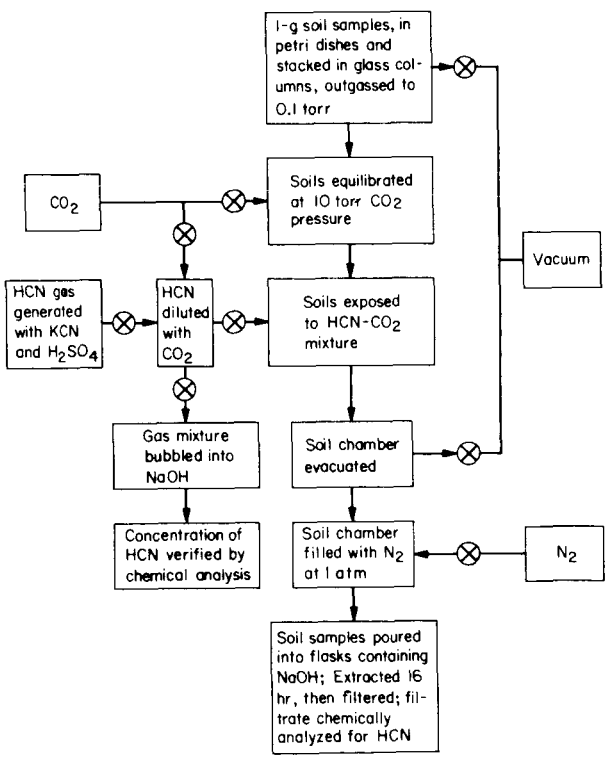

Figure 2.– Flow diagram of method used to study HCN sorption-desorption in soil.

Effect of HCN and NH_3 on the Anaerobic Respiration and Survival of Microorganisms in Terrestrial and Simulated Martian Soils

Anaerobic respiration studies.– The 80–100 mesh fractions of eight air-dried California soils were selected for these studies. Ground Colorado basaltic rock (Hazen Company) of varying particle size (furnished by Martin-Marietta Corporation) served as a simulated Martian soil (table 1).

Several of the terrestrial soils and the Hazen soil in petri dishes were exposed to: (1) 100 percent CO_2, (2) 500 ppm HCN in CO_2, (3) 5000 ppm HCN in CO_2, and (4) 5000 ppm HCN/30 percent NH_3 in CO_2 for 21 hours using the desiccator chambers and gas manifold system described previously. All soils in the anaerobic respiration and survival studies were outgassed to 0.1 torr and then brought to 10 torr CO_2 pressure and equilibrated before admission of the test gases at 10 torr. Commercial anhydrous NH_3, cp grade (99 percent) was used. The soils were held under dry N_2 for 4 days before respiration studies were begun (Viking samples are acquired 4 days after landing).

TABLE 1.– COLONY FORMING UNITS OF CONTROL SOILS

Soil sample	Soil description	Number of CFU per g of 80/100 mesh soil	
		Aerobic	Anaerobic
DV-3S	Death Valley sand dune	1.47×10^{4}	1.96×10^{2}
Aiken	Timberland soil, reddish, acid clay, loam and clay	1.71×10^{6}	1.37×10^{5}
Waukena	Saline and alkaline sandy loam	1.60×10^{5}	1.07×10^{3}
Salinas loam	Agricultural soil loam with calcareous subsoil	2.03×10^{7}	7.92×10^{5}
Staten Island peaty muck	High organic soil, poorly drained, acid in reaction	1.25×10^{7}	7.22×10^{5}
Siskiyou	Acid timberland soil, sandy loam, high rainfall	1.76×10^{6}	1.48×10^{5}
Bowers Clay 4	Grey alluvial clay	1.33×10^{7}	1.44×10^{4}
Holtville	Light colored, calcareous, alluvial soil	3.44×10^{7}	5.82×10^{4}
Hazen	Ground continental basalts (simulated Martian soil)	6.03×10^{4}	1.00×10^{3}

The gas chromatographic system used for the study of microbial respiration has been described by Carle (ref. 5). The 100–μl gas sampling loop was evacuated with a 5-cfm pump before a sample gas was obtained. A pair of 7.5 meter capillary columns (Porapak Q-A.W.) and a microbead detector were operated at room temperature. As Carle points out, there is a proportional loss of pressure in the incubation cell each time a sample is withdrawn (about 11 percent) because of the small volume of the incubation cell. Normalizing to krypton, which is present as an internal standard in the initial gas fill mixture, largely compensates for this loss. Oxygen, when present, was resolved in the chromatogram and used as a means of checking cell leakage.

The chambers for testing soils for gas changes are shown in figure 3. One-fourth ml of the test soil and 2 ml of medium M–4 (ref. 6) (modified by increasing KNO_3 from 1×10^{-3} M to 3×10^{-3} M and adding 1×10^{-2} M NH_4Cl) were put in each cell. The cells were checked for leaks, evacuated, and then filled to a pressure of 1 atm with a gas mixture containing 1.39 percent CO_2, 1.38 percent Kr, and the balance, helium (containing <25 ppm O_2 and N_2). The control samples were equivalent amounts of the unexposed soils plus medium and gas mixture, and soils that had been sterilized by dry heat (3 hours at 170°–180° C) plus medium and gas mixture. The results obtained with the sterilized soils were subtracted from the results obtained with the test soils.

The incubation cells were sampled when filled (0 days), then daily for the first 5 days and thereafter on alternate days through the thirteenth day. On the fourteenth day the cells were sampled before the medium was removed and 2 ml of fresh medium were added to each cell. The cells were then evacuated and filled with the gas mixture, and the sampling cycle was repeated for a second 2-week period.

Survival studies.– A set of 1-g samples of the eight California soils and the simulated Martian soil (Hazen) was exposed to 500 ppm HCN in 10 torr CO_2 for 80 minutes (fig. 4). The exposure time was increased to 21 hours with a second set of soil samples. Then three sets of the same soils were exposed to (1) 100 percent CO_2, (2) 30 percent NH_3 in CO_2, and (3) 500 ppm HCN in CO_2 for 21 hours followed by a 4-day period under dry nitrogen. The 1-g aliquots of all of the exposed soils were then plated.

Test and control soils in the survival studies were plated aerobically and anaerobically to determine their microbial populations. The 1-g portions of soil were placed in tubes containing 10 ml thioglycollate broth. The tube contents were mixed for 10 seconds with a Vortex Mixer, and serial tenfold dilutions were made in trypticase soy broth and thioglycollate broth. The soil dilutions were plated in triplicate using trypticase soy medium (2 percent agar) and thioglycollate medium (1 percent agar). The aerobic plates (trypticase soy) were inoculated by depositing 0.1 ml of the soil dilution on the surface of the agar and distributing the liquid with a sterile L-shaped glass rod. Anaerobic pour plates were made using 1 ml of the soil dilution and approximately 20 ml of molten thioglycollate agar. All plates were incubated at room temperature for 72 hours. The anaerobic plates were incubated in Brewer jars in a hydrogen-carbon dioxide environment (Bioquest). The colony forming units (CFU) were counted and the test soil results were compared with the number of CFU obtained from the control soils. All data were evaluated using Student's paired *t* test (refs. 7 and 8).

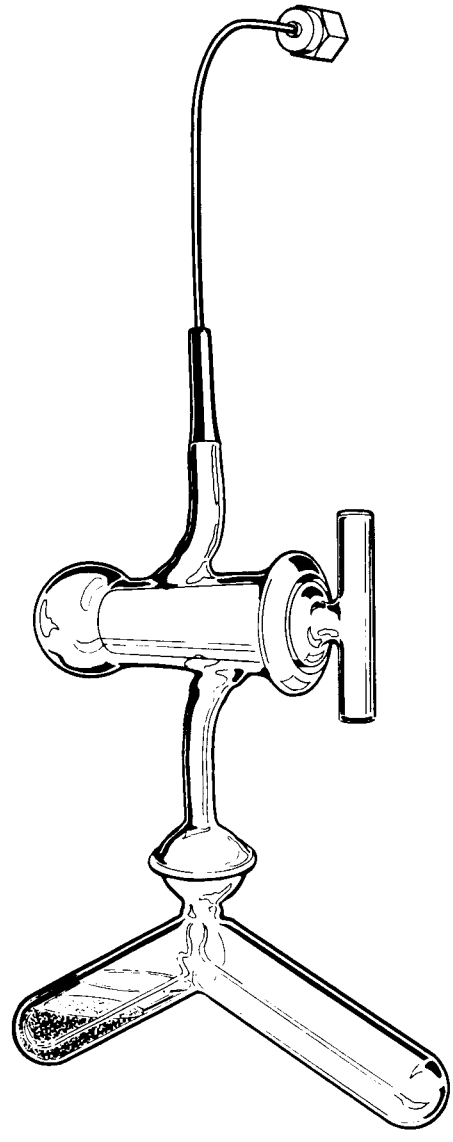

Figure 3.– Gas analysis cell, 9 ml in volume.

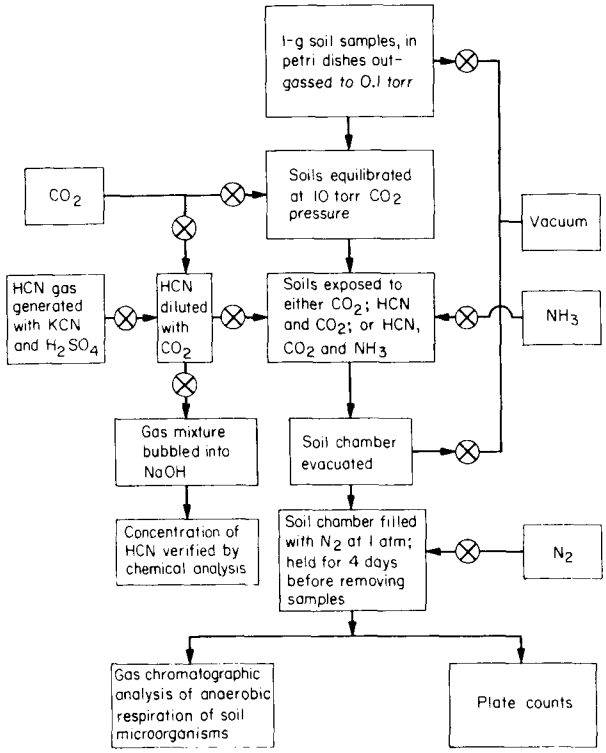

Figure 4.– Flow diagram of method used to study the effect of HCN and NH_3 on microorganisms in soil.

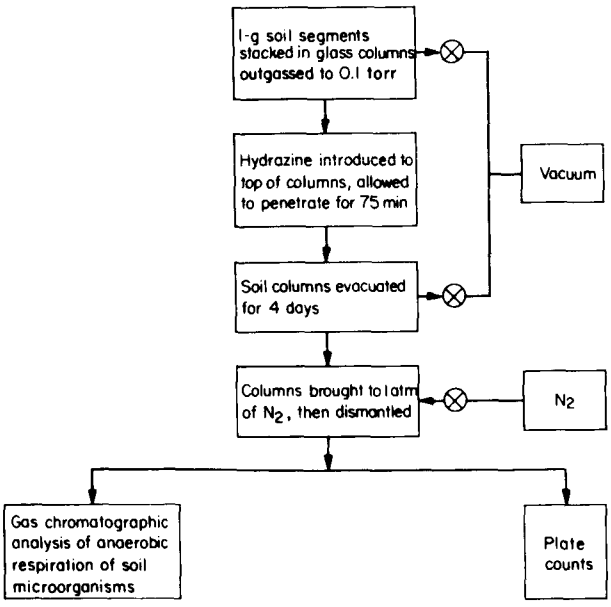

Figure 5.– Flow diagram of method used to study the effect of liquid hydrazine fuel on soil microorganisms.

Effect of Liquid Hydrazine Fuel on the Anaerobic Respiration and Survival of Soil Microorganisms

Anaerobic respiration studies.– Glass columns, partly filled with 1-g segments of Hazen and Bowers Clay 4 soil, were prepared in the same manner as those used in the HCN sorption experiments. The columns were made in duplicate to provide material for both the soil respiration and microbial survival studies (fig. 5). The soil columns were outgassed from both ends for several hours. The mil-spec grade N_2H_4 was furnished by Martin-Marietta Corporation. The hydrazine (0.25–0.30 ml) was introduced to the top of the soil column by a hypodermic needle through a silicone rubber septum. A steel needle valve attached to the hypodermic needle provided the necessary control for fluid addition. The hydrazine was allowed to penetrate into the top of the soil column and remain there for 75 minutes. Throughout the exposure period the soil was observed. A top portion was totally immersed in liquid hydrazine. A sharp border separated this phase from a second one, which was dark in appearance and faded down the length of the column to where the soil showed no change from its original shade. The soils were described as wet, damp or dry (table 2). At termination, the hydrazine was pumped off at full vacuum from the top of the column. Wetness was no longer seen after a few minutes while a downward gradient of dampness persisted several hours. The columns were pumped for 4 days until the soil regained its original appearance. Control soils were also maintained under vacuum for 4 days. The soil columns were dismantled, and 0.25 ml of each test soil segment and control soil were placed in a gas analysis incubation cell with 2.0 ml of the modified M–4 medium. Soil respiration studies were carried out in the manner described earlier.

TABLE 2.– HYDRAZINE SPILL EXPERIMENT

Soil sample	Purpose	Total no. of segments	Segment no. wet[a]	Segment no. damp[a]	Segment no. dry[a]
Hazen	Plating	8	1	2	3-8
Hazen	Respiration studies	8	1,2	3, (4)[b]	(4)[b], 5-8
Bowers Clay 4	Plating	6	(1)[b]	(1)[b]	2-6
Bowers Clay 4	Respiration studies	6	1	(2)[b]	(2)[b], 3-6

[a]The numbers in the last 3 columns refer to specific segments which have been numbered 1 to 8, from the top to the bottom of each column.

[b]Partially.

Survival studies.– The duplicate columns of Hazen and Bowers Clay 4 soils exposed to liquid hydrazine were dismantled and each 1-g segment and 1-g aliquots of control soils were plated according to the procedure described earlier.

Effect of Rocket Engine Exhaust Residues on Cultures of Microorganisms

The Martin-Marietta Corporation conducted the test firings in a CO_2 atmosphere at the Manned Spacecraft Center's White Sands Test Facility (MSC-WSTF). A full-size prototype Viking monopropellant vernier engine was used. Sterile Pyrex glass rods, 4 mm in diameter and 30.5 cm long, were marked to indicate direction of the flow of the engine exhaust. These were secured in a vertical position in traps in line with the flow of the rocket engine exhaust during firing. After the engine firings, the rods, in Teflon racks designed to hold each rod in a separate stationary position, were placed in sterile containers for shipment to Ames Research Center (fig. 6).

Experiments were performed with Pyrex glass rods from two engine firings and a control set of rods that had been subjected to the cleaning procedures preceding the firing test. When the containers holding the test rods were opened, the odor of ammonia was intense. The rods that had been held in Trap A, nearest the rocket, were coated with a white powder more concentrated on the ends than on the central area. The rods from Trap B, farthest from the rocket, were coated with a smooth yellow-brown material. This substance appeared most heavily concentrated at the end farthest from the exhaust plume.

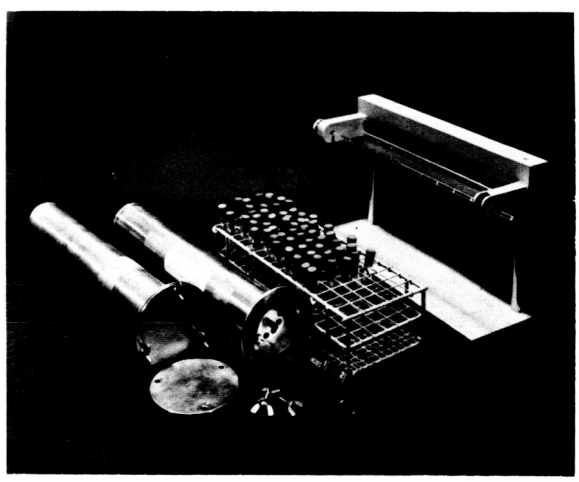

Figure 6.– Shipping containers and advancing device for Pyrex rods.

The individual glass rods were transferred aseptically to a sterilized device (fig. 6) designed to hold and advance the rods so that 1-in. segments could be cut with a sterile crimping tool. The segments were transferred with sterile forceps into test tubes (1 cm × 7.5 cm) containing 2 ml of broth medium. The tubes were plugged, then shaken on a rotary shaker for 2 hours (table 3). Three serial four-fold dilutions were made from each tube (fig. 7).

TABLE 3.– MICROORGANISMS USED IN ASSAY OF ROCKET BLAST RESIDUES COLLECTED ON GLASS RODS

Organism	Description	Growth medium	Incubation conditions, 30° C	Optimum plate incubation time, days
Scenedesmus obliquus	Green alga	Bishop's medium[a]	Under incandescent light	7-10
Candida lipolytica	Yeast	Trypticase soy	Aerobic	2
Bacillus subtilis	Aerobic sporeforming bacillus	Trypticase soy	Aerobic	2
Rhodospirillum rubrum	Photosynthetic spirillum	Van Niel's medium[b]	Under incandescent light	6-7
Clostridium acetobutylicum	Anaerobic sporeforming bacillus	Thioglycollate	Anaerobic	2
Escherichia coli	Facultative aerobic bacillus	Trypticase soy	Aerobic	2
Chlorella pyrenoidosa	Green alga	Chlorella medium[c]	Under incandescent light	7-10
Saccharomyces cerevisiae	Yeast	Trypticase soy	Aerobic	2
Sarcina lutea	Aerobic coccus (packets)	Trypticase soy	Aerobic	2-3
Rhodopseudomonas palustris	Photosynthetic bacillus	Van Niel's medium[b]	Under incandescent light	6-7
Clostridium butyricum	Anaerobic sporeforming bacillus	Thioglycollate	Anaerobic	2
Clostridium pasteurianum	Anaerobic sporeforming bacillus	Thioglycollate	Anaerobic	2

[a]Appendix A.
[b]Appendix B.
[c]Ref. 9.

Twelve microorganisms were selected to test the effect of rocket exhaust residues: two algae, *Chlorella pyrenoidosa* (ref. 9), and *Scenedesmus obliquus* (appendix A), two yeasts, *Saccharomyces cerevisiae*, and *Candida lipolytica;* three anaerobic bacteria, *Clostridium butyricum, Clostridium acetobutylicum,* and *Clostridium pasteurianum;* two photosynthetic bacteria (appendix B),

Rhodospirillum rubrum, and *Rhodopseudomonas palustris*; and three heterotrophic bacteria, *Bacillus subtilis, Escherichia coli,* and *Sarcina lutea.*

All tubes, including controls, were inoculated with 0.1 ml of a diluted broth culture of the test organism containing approximately 5×10^4 cells/ml. The diluted inoculum was plated to determine the number of viable cells introduced into each tube. The inoculated tubes were incubated at 30° C for 48 hours under conditions most favorable for the growth of the particular test organism (table 3). Triplicate tenfold serial dilution plates were made from each broth tube. Spread plates were used for aerobes and pour plates for anaerobes. After optimum incubation at 30° C (table 3), the CFU were counted and compared with the number of CFU in the controls.

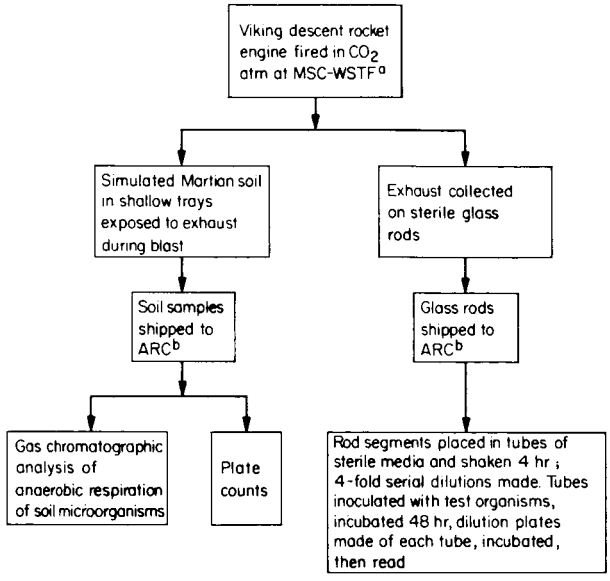

Figure 7.– Flow diagram of method used to study the effect of rocket engine exhaust on pure cultures of microorganisms and microorganisms in simulated Martian soil.

Effect of Rocket Engine Exhaust on the Anaerobic Respiration of the Microorganisms Within Simulated Martian Soil

Shallow beds of simulated Martian soil (Hazen) were placed in the flow of the rocket engine exhaust plume. Soil samples were obtained from three Viking lander retrorocket engine firings. The first and second firings utilized purified hydrazine fuel and an 18-nozzle cap to divert the engine exhaust. The last firing used mil-spec hydrazine fuel, which has been found to form HCN in low concentrations, and the same nozzle cap. Control soil samples from each test bed were also obtained.

The control soils and the soil samples from the three engine firing tests were used for gas chromatographic analyses of soil atmospheres utilizing the method described earlier.

RESULTS AND DISCUSSION

Sorption-desorption of HCN in Soil

Table 4 lists the amounts of HCN measured in evacuated soils exposed to 10-torr CO_2 containing HCN at two concentrations. The quantities retained after a half-hour pumping period following exposure to test gas are also listed. Figure 8 shows the observed depth of penetration

TABLE 4.– MICROGRAMS OF HCN SORBED/G OF VACUUM-DRIED SOIL IN EQUILIBRATED 10-TORR CO_2 SYSTEMS[a]

μg HCN sorbed per g vacuum-dried soil	Soil samples–group I			Soil samples–group II			Ignited soil samples[b]		
	Holt	Bowers	Aiken	Holt	Bowers	Aiken	Holt	Bowers	Aiken
Available HCN[c]	700[d]	700	700	77	77	77	700	700	700
Sorbed HCN[e]	28	112	188	9	57	157	151	471	607
Retention HCN[f]	5	12	68	1	4	13	6	9	13

[a]Soils were grouped together for exposure to HCN.

[b]Soils were heated from room temperature to 500° C (2 hr.) then removed from the oven.

[c]Available HCN is given per g of each soil in a group.

[d]700 μg of HCN are equivalent to 500 ppm b.v. of a 10-torr atmosphere in a 1000-m tall column which is 1 cm^2 in cross-section.

[e]The sorbed amount is the gross uptake within 2 hr in a static condition.

[f]Retention indicates quasi-permanent sorption not reversible by pumping for 30 min.

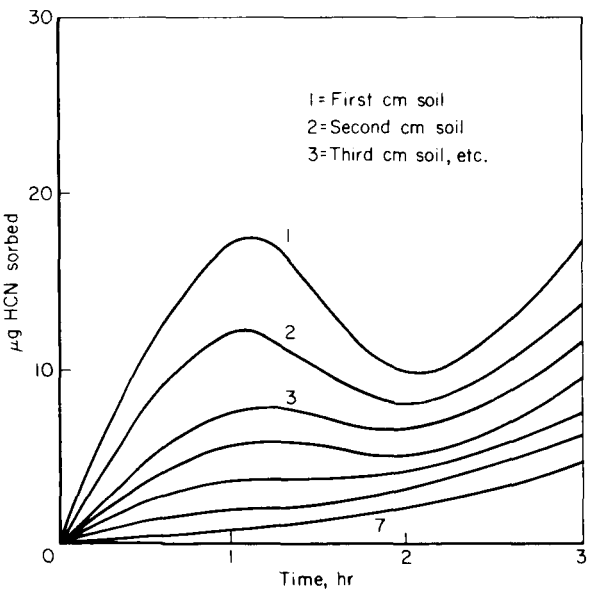

Figure 8.–Absorption of HCN by successive layers of Siskiyou soil (100–120 mesh) equilibrated at a 10-torr CO_2 pressure.
[System simulates a 10–m atmospheric column above a surface area of 1 cm^2; the available HCN is 70 ug or 500 ppm bv.]

with time by HCN into equilibrated soil. In this experiment, the CO_2–HCN mixture from a reservoir was admitted to a soil column that had been previously outgassed but then brought to 10 torr CO_2. The HCN under these conditions rapidly entered the first few centimeters of a 100–120 mesh soil column. Figure 9 illustrates the desorption of HCN; this desorption was effected by a gas sweep at the 10 torr level rather than a pumpout condition.

Effect of HCN and NH_3 on the Anaerobic Respiration and Survival of Microorganisms in Terrestrial and Simulated Martian Soils

Anaerobic respiration studies.– The results of the gas chromatographic analyses are shown in tables 5 through 10. In most soils the effect of 500 ppm HCN in CO_2 was inconclusive. Some soils exhibited slight inhibition of anaerobic respiration while others appeared to display some enhancement of gas production. Most of the soils tested manifested some inhibition of anaerobic respiration when exposed to 5000 ppm HCN in CO_2, but in no case was gas production eliminated. There appeared a partial reversal of this repression when 30 percent NH_3 was present.

Survival studies.– Some of the results are shown in table 11. There was very little difference between the percent growth obtained from soils exposed to 500 ppm HCN in CO_2 and that obtained from soils exposed only to 100 percent CO_2. The growth of aerobic microorganisms in Hazen, DV3-S and Salinas Loam soils exposed to HCN was less than that in the CO_2 control, but in no case was the indigenous aerobic population completely destroyed. There seemed to be an alteration of some soil populations after exposure to 30 percent NH_3 in CO_2 and an enhancement of microbial growth.

A review of the literature indicates that the effect of HCN on microorganisms is extremely variable, but that microorganisms in terrestrial soils exposed to the concentration of HCN expected in the Viking terminal engine exhaust would probably not be destroyed (refs. 10–22). The results of this study appear to agree with these findings. The literature concerning the effect of gaseous NH_3 on soil populations is scanty. It appears to have a detrimental effect on Nitrobacter (refs. 23–25). Liquid ammonia seems to destroy soil microorganisms, but the original population eventually reappears (ref. 26).

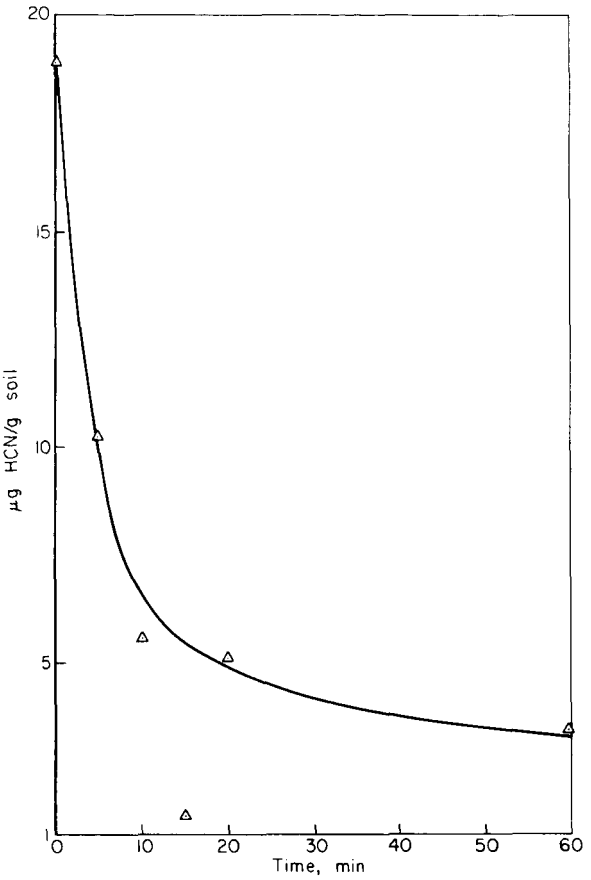

Figure 9.–Desorption of HCN from a 7 mm layer of Siskiyou soil 100–120 mesh at a velocity of 1 cu-ft/min CO_2 and a 10 torr pressure.

Effect of Liquid Hydrazine Fuel on the Anaerobic Respiration and Survival of Soil Microorganisms

Anaerobic respiration studies.– The hydrazine-treated Hazen soil column segments did not evidence any production of H_2 or CO_2, the gases produced normally by the indigenous population of the soil. The Bowers Clay 4 column displayed complete inhibition of H_2, CH_4 and CO_2 production in the first four segments, although only the first had been wet by the hydrazine fuel. The fifth and sixth segments both demonstrated production of these gases; the fifth segment, however, exhibited less than did the vacuum control. Nitrogen production was greater in those segments adversely affected by the liquid hydrazine than in the control (tables 12 and 13). The initial values for CO_2 in the gas analysis incubation cells containing Bowers Clay 4 were abnormally low. This soil has a high organic content and we conclude that hydrazine (but not ammonia gas which was used in other experiments) will aminate and iminate some of the organic material. The resulting basic functions will bind some of the carbon dioxide initially supplied or subsequently produced, thus creating a lag in the observed CO_2 readings.

TABLE 5.– RESPIRATORY GAS PRODUCTION BY MICROORGANISMS IN STATEN ISLAND PEATY MUCK SOIL EXPOSED TO TEST GASES

Gas produced	21-Hr gas exposure	Cycle one				Cycle two			
		Lag time, days	Maximum gas output		Gas at termination, nmoles	Lag time, days	Maximum gas output		Gas at termination, nmoles
			Time, days	Gas, nmoles			Time, days	Gas, nmoles	
H_2	100% CO_2	2	3	213	1.68	1	2	258	3.63
	500 ppm HCN	2	3	298	2.41	1	2	276	9.71
	5000 ppm HCN	2	5	245	4.80	1	3	89.5	16.5
	5000 ppm HCN, 30% NH_3	2	7	34.8	22 2	1	3	18.2	0
N_2	100% CO_2	1	4	25.7	24.4	1	2	1.80	0
	500 ppm HCN	1	4	21.5	18.8	1	2	2.02	0
	5000 ppm HCN	1	7	5.69	2.65	1	14	5.31	5.31
	5000 ppm HCN, 30% NH_3	1	14	1.70	1.70	1	9	1.25	0.834
CH_4	100% CO_2	6	14	96.2	96.2	1	14	1646	1646
	500 ppm HCN	6	14	34.6	34.6	1	14	936	936
	5000 ppm HCN	7	14	3.52	3.52	3	14	46.3	46.3
	5000 ppm HCN, 30% NH_3	7	14	2.92	2.92	3	14	600	600
CO_2	100% CO_2	1	14	1228	1228	1	14	1503	1503
	500 ppm HCN	1	14	1213	1213	1	14	1527	1527
	5000 ppm HCN	1	14	552	552	1	14	362	362
	5000 ppm HCN, 30% NH_3	2	14	482	482	1	14	737	737
N_2O	100% CO_2	2	2	Trace	0			0	0
	500 ppm HCN	2	2	Trace	0			0	0
	5000 ppm HCN	2	2	Trace	0			0	0
	5000 ppm HCN, 30% NH_3			0	0	2	2	Trace	0

TABLE 6.– RESPIRATORY GAS PRODUCTION BY MICROORGANISMS IN DV3-S SOIL EXPOSED TO TEST GASES

Gas produced	21-Hr gas exposure	Cycle one				Cycle two			
		Lag time, days	Maximum gas output		Gas at termination, nmoles	Lag time, days	Maximum gas output		Gas at termination, nmoles
			Time, days	Gas, nmoles			Time, days	Gas, nmoles	
H_2	100% CO_2	7	14	556	556	1	14	267	267
	500 ppm HCN			0	0	7	14	214	214
	5000 ppm HCN	9	14	245	245	1	12	108	94
	5000 ppm HCN, 30% NH_3	7	14	50.5	50.5	1	14	185	185
N_2	100% CO_2	5	14	13.3	13.3	1	14	13.1	13.1
	500 ppm HCN	2	14	2.32	2.32	1	14	11.3	11.3
	5000 ppm HCN	1	14	2.90	2.90	1	7	1.40	0.47
	5000 ppm HCN, 30% NH_3	3	12	1.37	1.17	1	14	2.39	2.39
CH_4	100% CO_2			0	0			0	0
	500 ppm HCN			0	0			0	0
	5000 ppm HCN			0	0			0	0
	5000 ppm HCN, 30% NH_3			0	0			0	0
CO_2	100% CO_2	5	14	832	832	1	14	701	701
	500 ppm HCN	9	14	13.1	13.1	1	14	485	485
	5000 ppm HCN	9	14	235	235	1	14	162	162
	5000 ppm HCN, 30% NH_3	3	14	391	391	2	14	492	492
N_2O	100% CO_2			0	0			0	0
	500 ppm HCN			0	0			0	0
	5000 ppm HCN			0	0			0	0
	5000 ppm HCN, 30% NH_3			0	0			0	0

TABLE 7.– RESPIRATORY GAS PRODUCTION BY MICROORGANISMS IN HAZEN SOIL EXPOSED TO TEST GASES

Gas produced	21-Hr gas exposure	Cycle one				Cycle two			
		Lag time, days	Maximum gas output		Gas at termination, nmoles	Lag time, days	Maximum gas output		Gas at termination, nmoles
			Time, days	Gas, nmoles			Time, days	Gas, nmoles	
H_2	100% CO_2	6	14	1645	1645	1	14	215	215
	500 ppm HCN	6	12	180	178	1	14	124	124
	5000 ppm HCN	5	14	76.6	76.6	2	14	94.3	94.3
	5000 ppm HCN, 30% NH_3	5	14	790	790	1	14	159	159
N_2	100% CO_2	2	6-12	58	58	1	14	19.6	19.6
	500 ppm HCN	2	6-12	73	69	1	14	17.7	17.7
	5000 ppm HCN	1	14	12.6	12.6	1	14	14.0	14.0
	5000 ppm HCN, 30% NH_3	1	12	16.4	15.9	1	14	27.7	27.7
CH_4	100% CO_2			0	0			0	0
	500 ppm HCN			0	0			0	0
	5000 ppm HCN			0	0			0	0
	5000 ppm HCN, 30% NH_3			0	0			0	0
CO_2	100% CO_2	2	14	1734	1734	1	14	1251	1251
	500 ppm HCN	2	14	1286	1286	1	14	726	726
	5000 ppm HCN	5	14	303	303	1	14	288	288
	5000 ppm HCN, 30% NH_3	5	14	845	845	1	14	637	637
N_2O	100% CO_2			0	0	1	3-10	4	3.5
	500 ppm HCN			0	0	2	12	4.5	3.4
	5000 ppm HCN			0	0			0	0
	5000 ppm HCN, 30% NH_3	5	12	11.1	11.0	12	12	1.67	0

TABLE 8.– RESPIRATORY GAS PRODUCTION BY MICROORGANISMS IN AIKEN SOIL EXPOSED TO TEST GASES

Gas produced	21-Hr gas exposure	Cycle one				Cycle two			
		Lag time, days	Maximum gas output		Gas at termination, nmoles	Lag time, days	Maximum gas output		Gas at termination, nmoles
			Time, days	Gas, nmoles			Time, days	Gas, nmoles	
H_2	100% CO_2	3	6	879	371	1	[a]3; 14	[a]17.2; 11.4	11.4
	500 ppm HCN	2	6	471	237	1	[a]3; 12	[a]11.7; 13.1	6.75
	5000 ppm HCN	3	5	294	272	1	3	15.8	3.81
	5000 ppm HCN, 30% NH_3	(b)	(b)	(b)	(b)	(b)	(b)	(b)	(b)
N_2	100% CO_2	1	14	2.97	2.97	1	14	1.99	1.99
	500 ppm HCN	1	14	11.34	11.34	1	12	3.62	0.68
	5000 ppm HCN	1	14	6.82	6.82	1	14	0.25	0.25
	5000 ppm HCN, 30% NH_3	(b)	(b)	(b)	(b)	(b)	(b)	(b)	(b)
CH_4	100% CO_2			0	0			0	0
	500 ppm HCN			0	0			0	0
	5000 ppm HCN			0	0			0	0
	5000 ppm HCN, 30% NH_3	(b)	(b)	(b)	(b)	(b)	(b)	(b)	(b)
CO_2	100% CO_2	2	14	1327	1327	1	14	1361	1361
	500 ppm HCN	2	14	1187	1187	1	14	1263	1263
	5000 ppm HCN	3	14	487	487	1	14	260	260
	5000 ppm HCN, 30% NH_3	(b)	(b)	(b)	(b)	(b)	(b)	(b)	(b)
N_2O	100% CO_2			0	0			0	0
	500 ppm HCN			0	0			0	0
	5000 ppm HCN			0	0			0	0
	5000 ppm HCN, 30% NH_3	(b)	(b)	(b)	(b)	(b)	(b)	(b)	(b)

[a]Two subcycles H_2.
[b]Not tested.

TABLE 9.– RESPIRATORY GAS PRODUCTION BY MICROORGANISMS IN WAUKENA SOIL EXPOSED TO TEST GASES

Gas produced	21-Hr gas exposure	Cycle one				Cycle two			
		Lag time, days	Maximum gas output		Gas at termination, nmoles	Lag time, days	Maximum gas output		Gas at termination, nmoles
			Time, days	Gas, nmoles			Time, days	Gas, nmoles	
H_2	100% CO_2	9	14	65.5	65.5	1	12	107	73.3
	500 ppm HCN	9	14	404	404	1	10	44.5	34.4
	5000 ppm HCN	7	9	47.3	44.3	1	14	9.36	9.36
	5000 ppm HCN, 30% NH_3	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)
N_2	100% CO_2	1	14	0.66	0.66	1	10	0.97	0.96
	500 ppm HCN	1	14	0.61	0.61	1	12	1.87	1.11
	5000 ppm HCN	1	14	0.95	0.95	1	14	0.63	0.63
	5000 ppm HCN, 30% NH_3	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)
CH_4	100% CO_2			0	0			0	0
	500 ppm HCN			0	0	6	14	0.76	0.76
	5000 ppm HCN			0	0			0	0
	5000 ppm HCN, 30% NH_3	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)
CO_2	100% CO_2	3	14	774	774	1	14	1487	1487
	500 ppm HCN	3	14	1677	1677	1	14	1421	1421
	5000 ppm HCN	5	14	520	520	1	14	218	218
	5000 ppm HCN, 30% NH_3	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)
N_2O	100% CO_2			0	0			0	0
	500 ppm HCN			0	0			0	0
	5000 ppm HCN			0	0			0	0
	5000 ppm HCN, 30% NH_3	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)

[a]Not tested.

TABLE 10.– RESPIRATORY GAS PRODUCTION BY MICROORGANISMS IN BOWERS CLAY 4 EXPOSED TO TEST GASES

Gas produced	21-Hr gas exposure	Cycle one				Cycle two			
		Lag time, days	Maximum gas output		Gas at termination, nmoles	Lag time, days	Maximum gas output		Gas at termination, nmoles
			Time, days	Gas, nmoles			Time, days	Gas, nmoles	
H_2	100% CO_2	2	3	31.4	0	1	[a]4; 14	[a]38.0; 22.4	22.4
	500 ppm HCN	2	3	37.2	0	1	[a]4; 12	[a]14.8; 12.1	5.84
	5000 ppm HCN	2	5	46.0	5.19	1	1	7.67	3.03
	5000 ppm HCN, 30% NH_3	(b)	(b)	(b)	(b)	(b)	(b)	(b)	(b)
N_2	100% CO_2	1	14	2.32	2.32	1	14	2.01	2.01
	500 ppm HCN	1	14	0.57	0.57	1	12	2.47	1.45
	5000 ppm HCN	1	12	2.29	1.66	1	14	0.20	0.20
	5000 ppm HCN, 30% NH_3	(b)	(b)	(b)	(b)	(b)	(b)	(b)	(b)
CH_4	100% CO_2	12	14	12.6	12.6	2	14	66.4	66.4
	500 ppm HCN	9	14	29.7	29.7	1	14	1351	1351
	5000 ppm HCN	12	14	1.48	1.48	1	14	230	230
	5000 ppm HCN, 30% NH_3	(b)	(b)	(b)	(b)	(b)	(b)	(b)	(b)
CO_2	100% CO_2	2	14	1401	1401	1	14	1205	1205
	500 ppm HCN	2	14	1189	1189	1	14	1384	1384
	5000 ppm HCN	2	14	537	537	1	14	352	352
	5000 ppm HCN, 30% NH_3	(b)	(b)	(b)	(b)	(b)	(b)	(b)	(b)
N_2O	100% CO_2			0	0			0	0
	500 ppm HCN			0	0			0	0
	5000 ppm HCN			0	0			0	0
	5000 ppm HCN, 30% NH_3	(b)	(b)	(b)	(b)	(b)	(b)	(b)	(b)

[a]Two subcycles H_2.
[b]Not tested.

TABLE 11.– SURVIVAL OF AEROBIC AND ANAEROBIC INDIGENOUS SOIL POPULATIONS FOLLOWING EXPOSURE TO VARIOUS TEST GASES

Soil sample, 1g, 80-100 mesh size	Percent growth following 21-hr exposure to 500 ppm HCN in CO_2 [a]		Percent growth following 21-hr exposure to 100 percent CO_2 [a]		Percent growth following 21-hr exposure to 30 percent NH_3 in CO_2 [a]	
	Aerobic	Anaerobic	Aerobic	Anaerobic	Aerobic	Anaerobic
Hazen[b]	57.7[c]	110.0	109.3	105.0	99.2	101.0
DV 3-S	59.2[c]	15.3[c]	72.8	16.8[c]	59.0	77.6
Aiken	179.8[c]	105.1	90.1	61.1	429.8[c]	375.2[c]
Waukena	70.0	64.0[c]	80.0	46.9[c]	51.8	94.4
Salinas loam	28.5[c]	13.6[c]	117.7	18.2[c]	366.0[c]	227.3[c]
Staten Island peaty muck	105.6	79.9	93.6	108.0	96.8	200.8[c]
Siskiyou	108.0	162.8[c]	126.1	148.6	101.1	672.7[c]
Bowers clay 4	62.9[c]	139.6[c]	42.2[c]	111.1	113.5	95.1
Holtville	72.1	123.7	81.4	140.0	523.3[c]	84.0

[a]Percent growth calculated by letting the amount of growth in the control equal 100 percent.
[b]Random size.
[c]Significant difference between the mean of the control and the mean of the test at the 1 percent level.

TABLE 12.– RESPIRATORY GAS PRODUCTION BY MICROORGANISMS IN SEGMENTS OF HAZEN SOIL COLUMNS EXPOSED TO LIQUID HYDRAZINE

Gas produced		Lag time, days	Cycle one			Cycle two			
			Maximum gas output		Gas at termination, nmoles	Lag time, days	Maximum gas output		Gas at termination, nmoles
			Time, days	Gas, nmoles			Time, days	Gas, nmoles	
H_2	Control	3	12	1428	1422	1	12	2313	2299
	Segment 1			0	0			0	0
	Segment 6			0	0			0	0
N_2	Control	1	12	10.6	10.6	1	3	48	46.9
	Segment 1	1	14	13.7	13.7	1	14	7	7
	Segment 6	1	14	18.5	1	1	14	6.3	6.3
CH_4	Control			0	0			0	0
	Segment 1			0	0			0	0
	Segment 6			0	0			0	0
CO_2	Control	3	14	1069	1069	1	14	1541	1541
	Segment 1	5	14	10.5	10.5	9	14	76.6	76.6
	Segment 6	3	14	15	15	9	14	81.2	81.2
N_2O	Control			0	0			0	0
	Segment 1			0	0			0	0
	Segment 6			0	0			0	0

TABLE 13.– RESPIRATORY GAS PRODUCTION BY MICROORGANISMS IN SEGMENTS OF BOWERS CLAY 4 SOIL COLUMNS EXPOSED TO LIQUID HYDRAZINE

Gas produced		Cycle one				Cycle two			
		Lag time, days	Maximum gas output		Gas at termination, nmoles	Lag time, days	Maximum gas output		Gas at termination, nmoles
			Time, days	Gas, nmoles			Time, days	Gas, nmoles	
H_2	Control	2	3	216.5	14.6	1	2	40.5	0
	Segment 1			0	0			0	0
	Segment 6	2	2	186.7	9.1	1	2	30.3	0
N_2	Control	1	14	10.4	10.4	1	14	2.9	2.9
	Segment 1	1	14	24.1	24.1	1	14	166.5	166.5
	Segment 6	1	14	12.3	12.3	1	12	2.7	2.6
CH_4	Control	7	14	121	121	1	14	5092	5092
	Segment 1			0	0			0	0
	Segment 6	7	14	173.8	173.8	1	14	4319	4319
CO_2	Control	1	14	1717	1717	1	14	1961	1961
	Segment 1	14	14	46.9	46.9	2	14	22	22
	Segment 6	1	14	1848	1848	1	14	2650	2650
N_2O	Control			0	0			0	0
	Segment 1			0	0			0	0
	Segment 6			0	0			0	0

Survival studies.– Few, if any, microorganisms were isolated from the soil wetted by hydrazine (table 14). In Hazen soil, with increasing depths more colonies were formed, but at no depth did the number of CFU equal that in the control. In the Bowers Clay 4 column, only the bottom segment contained as many CFU as the control.

Effect of Rocket Engine Exhaust Residues on Cultures of Microorganisms

Residue from the Viking descent engine firings appeared to have little adverse effect upon microbial growth. Only three segments of a glass rod from Trap B, Rocket Test 005, using *Candida lipolytica* as a test organism, indicated any inhibition. This inhibition abated in the first dilution. These segments were from the center of the rod and were not the segments with the heaviest visible residue. Growth of a number of the test organisms appeared to be stimulated by the residue of the engine exhaust plume. These results are shown in tables 15 through 18.

Effect of Rocket Engine Exhaust on the Anaerobic Respiration of the Microorganisms in Simulated Martian Soil

No inhibition of microbial respiration by exposure of the simulated Martian soil to actual rocket engine exhaust is apparent. As evidenced by tables 19 through 22, microbial gas production in the control soils and in the test soils was virtually the same.

TABLE 14.– EFFECT OF MIL-SPEC LIQUID HYDRAZINE FUEL UPON MICROORGANISMS IN SOIL COLUMNS

Hazen soil, 1g, vacuum-dried for 4 days	Percent growth[a]		Bowers Clay 4 soil, 1g, vacuum-dried for 4 days	Percent growth[a]	
	Aerobic	Anaerobic		Aerobic	Anaerobic
Vacuum-dried control	85.5	46.1[c]	Vacuum-dried control	133.8	95.8
Segment [b]1	0[c]	0[c]	Segment 1	0.007[c]	0.116[c]
Segment 2	0[c]	0[c]	Segment 2	15.6[c]	36.1[c]
Segment 3	0[c]	0[c]	Segment 3	42.9[c]	82.6
Segment 4	0.347[c]	1.13[c]	Segment 4	63.4	89.8
Segment 5	0.576[c]	1.47[c]	Segment 5	80.3	100.7
Segment 6	3.72[c]	11.90[c]	Segment 6	92.1	101.4
Segment 7	8.23[c]	38.40[c]			
Segment 8	13.60[c]	42.90[c]			

[a]Percent growth calculated by letting the amount of growth in the non-vacuum-dried control equal 100 percent;
[b]Mil-spec liquid hydrazine fuel added to the top of column; segments numbered 1-6 or 1-8 from the top of the column to the bottom.
[c]Significant difference between the mean of the control and the mean of the test at the 1 percent level.

TABLE 15.– GROWTH EFFECTS OF ROCKET TEST 005 (TRAP B) EXHAUST RESIDUES ON MICROORGANISMS

Test organism	Percent growth in rod segments[a]											
	1	2	3	4	5	6	7	8	9	10	11	12
Bacillus subtilis, rod B-12	85.7	91.0	121.0	96.7	123.0	84.0	123.0	109.0	132.0	100.0	120.0	108.0
Candida lipolytica, rod B-3	53.6[b]	40.4[b]	48.8[b]	65.2[b]	0.102[b]	2.27[b]	0.543[b]	77.7[b]	63.0[b]	69.1[b]	74.6[b]	86.3
Candida lipolytica, rod B-3, 1:4 dil.	69.2	78.7	65.9	81.2	65.6	64.4	73.6	90.7	94.3	91.1	96.5	105.0
Rhodospirillum rubrum, rod B-4	99.3	101.4	88.5	91.0	89.9	99.3	82.7	88.5	94.2	84.2	97.2	100.0
Scenedesmus obliquus, rod B-5	88.8	82.4	82.9	86.5	85.9	88.2	87.1	84.7	84.7	98.2	91.8	86.5
Clostridium acetobutylicum, rod B-6	97.3	105.0	105.0	106.0	95.9	97.9	97.9	101.0	93.2	87.1	104.0	92.5

[a]Data derived from the first (undiluted) tube in each dilution series except as noted otherwise; percent growth calculated by letting the amount of growth in the control equal 100 percent; segments numbered 1-12 from the top of the rod to the bottom.
[b]Significant difference between the mean of the control and the mean of the test at the 1 percent level.

TABLE 16.– GROWTH OF MICROORGANISMS USING RODS FROM ROCKET TEST 006 (TRAP A) WHICH HAD BEEN SUBJECTED TO THE PRETEST CLEANING PROCEDURE BUT NOT THE ROCKET EXHAUST

Test organism	Percent growth in rod segments[a]											
	1	2	3	4	5	6	7	8	9	10	11	12
Chlorella pyrenoidosa, rod A-1	95.3	99.8	116.5	104.2	100.7	102.9	99.6	111.8	111.8	106.2	101.8	100.2
Sarcina lutea, rod A-2	87.0	103.9	113.5	109.8	87.4	98.5	114.1	103.7	105.0	118.0	104.8	101.7
Saccharomyces cerevisiae, rod A-5	104.9	105.7	107.4	109.8	100.8	106.6	104.9	104.1	100.8	104.1	105.7	105.7
Rhodopseudomonas palustris, rod A-6	104.6	111.6	113.0	93.4	106.9	101.2	99.6	107.0	102.2	102.3	103.5	103.0

[a]Data derived from the first (undiluted) tube in each dilution series; percent growth calculated by letting the amount of growth in the control equal 100 percent; segments numbered 1-12 from the top of the rod to the bottom; no significant difference between the mean of the control and the mean of the test at the 1 percent level in any of the above rod segments.

TABLE 17.– GROWTH OF MICROORGANISMS USING RODS FROM ROCKET TEST 006 (TRAP B) WHICH HAD BEEN SUBJECTED TO THE PRETEST CLEANING PROCEDURE BUT NOT THE ROCKET EXHAUST

Test organism	Percent growth in rod segments[a]											
	1	2	3	4	5	6	7	8	9	10	11	12
Sarcina lutea, rod B-2	115.6	88.8	96.5	115.6	103.7	110.6	117.2	106.2	103.1	119.7	104.2	118.9
Chlorella pyrenoidosa, rod B-3	95.1	107.4	100.2	105.2	103.2	99.8	105.2	97.5	97.1	104.4	102.5	92.3
Clostridium butyricum, rod B-4	109.3	106.0	85.8	106.0	101.6	101.6	100.0	105.5	109.3	114.2	98.1	103.3
Saccharomyces cerevisiae, rod B-5	109.1	111.6	101.7	100.8	107.4	105.8	105.0	105.0	106.6	104.1	105.0	104.1
Rhodopseudomonas palustris, rod B-6	102.1	108.0	109.2	107.2	98.5	92.7	101.9	91.8	103.3	93.3	100.3	97.2

[a]Data derived from the first (undiluted) tube in each dilution series; percent growth calculated by letting the amount of growth in the control equal 100 percent; segments numbered 1-12 from the top of the rod to the bottom; no significant difference between the mean of the control and the mean of the test at the 1 percent level in any of the above rod segments.

TABLE 18.– GROWTH EFFECTS OF ROCKET TEST 007 (TRAPS A AND B) EXHAUST RESIDUES ON MICROORGANISMS

Test organism	Percent growth in rod segments[a]											
	1	2	3	4	5	6	7	8	9	10	11	12
Escherichia coli, rod A-1	98.5	108.0	110.0	108.0	119.0	124.0	140.0	132.0	135.0	124.0	107.0	97.9
Bacillus subtilis, rod A-2	113.0	108.0	99.0	91.2	94.4	86.4	99.2	94.2	107.0	94.0	122.0	85.6
Candida lipolytica, rod A-3	98.4	94.3	114.9	94.3	95.3	136.9[b]	108.2	107.0	111.0	81.0	69.3[b]	102.3
Rhodospirillum rubrum, rod A-4	107.0	96.3	103.0	95.5	104.0	96.3	99.2	101.0	101.0	101.0	106.0	101.0
Scenedesmus obliquus, rod A-5	86.0	95.9	90.7	84.9	81.4	79.7[b]	79.7[b]	82.6[b]	79.7[b]	91.9	91.9	82.6
Clostribium acetobutylicum, rod A-5	92.7	101.0	101.0	86.8	92.7	106.0	94.7	102.0	103.0	84.8	94.1	94.7
Escherichia coli, rod B-1, trap B	82.2	85.3	94.6	88.5	87.6	108.1	100.5	89.8	88.0	104.5	119.0	100.5

[a]Data derived from the first (undiluted) tube in each dilution series; percent growth calculated by letting the amount of growth in the control equal 100 percent; segments numbered 1-12 from the top of the rod to the bottom.
[b]Significant difference between the mean of the control and the mean of the test at the 1 percent level.

TABLE 19.– RESPIRATORY GAS PRODUCTION BY MICROORGANISMS IN HAZEN SOIL (5 FT, TOP LEFT SAMPLE) EXPOSED TO THE VIKING DESCENT ENGINE EXHAUST, MAY 6, 1971[a]

Gas produced		Cycle one				Cycle two			
		Lag time, days	Maximum gas output		Gas at termination, nmoles	Lag time, days	Maximum gas output		Gas at termination, nmoles
			Time, days	Gas, nmoles			Time, days	Gas, nmoles	
H_2	Control	2	14	42.5	42.5	2	14	123.0	123.0
	Test	3	14	94.7	94.7	2	7	50.4	37.0
N_2	Control	1	14	8.4	8.4	1	9	10.3	10.1
	Test	1	14	16.3	16.3	1	7	30.3	26.2
CH_4	Control			0	0			0	0
	Test			0	0			0	0
CO_2	Control	2	14	450.0	450.0	1	14	549.0	549.0
	Test	3	14	395.0	395.0	1	14	392.0	392.0
N_2O	Control	3	7	2.3	1.5	9	9	0.9	0
	Test	12	12	1.2	0	1	2	2.2	0

[a]Purified hydrazine fuel was used.

TABLE 20.– RESPIRATORY GAS PRODUCTION BY MICROORGANISMS IN HAZEN SOIL (11 FT, TOP LEFT SAMPLE) EXPOSED TO THE VIKING DESCENT ENGINE EXHAUST, MAY 6, 1971[a]

Gas produced		Cycle one				Cycle two			
		Lag time, days	Maximum gas output		Gas at termination, nmoles	Lag time, days	Maximum gas output		Gas at termination, nmoles
			Time, days	Gas, nmoles			Time, days	Gas, nmoles	
H_2	Control	2	9	173.0	6.3	1	3	43.4	31.1
	Test	5	14	289.0	289.0	2	14	257.0	257.1
N_2	Control	1	14	17.5	17.5	1	14	24.0	24.0
	Test	1	5	33.6	24.1	1	7	29.0	28.4
CH_4	Control			0	0			0	0
	Test			0	0			0	0
CO_2	Control	2	14	921.0	921.0	1	14	694.0	694.0
	Test	2	14	726.0	726.0	1	14	789.0	789.0
N_2O	Control			0	0	1	2	3.6	1.6
	Test			0	0			0	0

[a]Purified hydrazine fuel was used.

TABLE 21.– RESPIRATORY GAS PRODUCTION BY MICROORGANISMS IN HAZEN SOIL (V.O. 5.5 FT SAMPLE) EXPOSED TO VIKING DESCENT ENGINE EXHAUST, JUNE 8, 1971[a]

Gas produced		Cycle one				Cycle two			
		Lag time, days	Maximum gas output		Gas at termination, nmoles	Lag time, days	Maximum gas output		Gas at termination, nmoles
			Time, days	Gas, nmoles			Time, days	Gas, nmoles	
H_2	Control	2	7	478.0	364.0	1	14	204.0	204.0
	Test	2	9	276.0	235.0	1	14	1153.0	1153.0
N_2	Control	1	9	15.8	14.3	1	14	88.2	88.2
	Test	1	7	7.3	7.1	1	7	41.2	40.5
CH_4	Control			0	0			0	0
	Test			0	0			0	0
CO_2	Control	2	14	1062.0	1062.0	2	14	715.0	715.0
	Test	2	14	513.0	513.0	2	14	809.0	809.0
N_2O	Control			0	0			0	0
	Test			0	0			0	0

[a]Purified hydrazine fuel was used.

TABLE 22.– RESPIRATORY GAS PRODUCTION BY MICROORGANISMS IN HAZEN SOIL (GL 5 FT SAMPLE) EXPOSED TO VIKING DESCENT ENGINE EXHAUST, JUNE 16, 1971[a]

Gas produced		Cycle one				Cycle two			
		Lag time, days	Maximum gas output		Gas at termination, nmoles	Lag time, days	Maximum gas output		Gas at termination, nmoles
			Time, days	Gas, nmoles			Time, days	Gas, nmoles	
H_2	Control	2	12	661.0	625.0	1	14	204.0	204.0
	Test	3	12	1145.0	1212.0	1	14	1153.0	1153.0
N_2	Control	1	14	7.0	7.0	1	14	14.0	89.3
	Test	1	7	44.5	44.2	1	7	42.3	41.6
CH_4	Control			0	0			0	0
	Test			0	0			0	0
CO_2	Control	2	14	782.0	782.0	2	14	716.0	716.0
	Test	2	14	1130.0	1130.0	2	14	808.0	808.0
N_2O	Control	5	5	2.2	0	9	9	0.7	0
	Test			0	0			0	0

[a]Mil-spec hydrazine fuel was used.

CONCLUSIONS

Physicochemical Aspects

A calculation of the dissipation rates for various gases under Martian conditions is not available. At Martian pressure, diffusion of even large molecules should be very rapid and a much greater number of compounds would volatilize from the landing site than in a terrestrial atmosphere. Ammonia or amines in absence of water react with carbon dioxide to form carbamates. The reaction depends on a minimum critical pressure and is reversible below that point. From preliminary observations we estimate that 50 torr would be required for the appearance of solid ammonium carbamate. Hydrogen cyanide, catalyzed by polar materials such as ammonia, is known to polymerize; only in quasi-condensed states (possibly under sorbed conditions) does this occur at a significant rate.

The sorption tests indicate that a dry soil takes up HCN in a loose manner. If water were trapped in the soil, a greater retention might be expected. Our test soils appeared to have a great capacity for reversible HCN sorption.

Two aspects of exposing soils to gas must be emphasized. First, because of the finite volume of any laboratory apparatus, a statement of gas concentration should be supplemented by an indication of the total amount of that component available to a given quantity of test soil, as shown in table 4. Secondly, in some experiments assorted soils were treated together in a single chamber and thus they became competitive.

Biological Findings

There has been justifiable concern about the effect of the products of the engine fuel on possible extraterrestrial life at the Martian landing site. This concern should be lessened by the results of these experiments. There were few or no detrimental effects on the terrestrial microorganisms tested, even at concentrations of rocket exhaust products in excess of those that would be released over the Martian surface. However, these tests have demonstrated that liquid hydrazine is extremely toxic.

Ames Research Center
National Aeronautics and Space Administration
Moffett Field, Calif. 94035, Aug. 1, 1972

APPENDIX A

SCENEDESMUS MEDIUM

[N. Bishop[1]]

KNO_3	0.809 g
NaCl	0.468 g
$Na_2HPO_4 .2H_2O$	0.178 g
$NaH_2PO_4 .2H_2O$	0.468 g
$CaCl_2 .6H_2O$	0.022 g
$MnCl_2 .4H_2O$	0.2 ml of a 0.1 percent solution
$MgSO_4 .7H_2O$	0.247 g
$FeSO_4 .7H_2O$	0.01 g
$ZnSO_4 .7H_2O$	0.1 ml of a 0.1 percent solution
Versene	0.02 g
Glucose	5.0 g
Yeast extract	2.5 g

Dissolve in 1 liter glass-distilled water. Add 2 percent agar for slants.

[1]Personal communication, Dr. Ellen Weaver, Department of Biology, San Jose State University, San Jose, California 95114.

APPENDIX B

VAN NIEL'S YEAST AGAR (REF. 27)

K_2HPO_4	1.0 g
$MgSO_4$	0.5 g
Yeast extract	10.0 g
Agar	20.0 g
Tap water	1000 ml

Adjust to pH 7.0–7.2

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16. Abstract

There has been concern about the effect of the products of the Viking terminal descent engine fuel upon possible extraterrestrial life at the Martian landing site. The effects of the engine exhaust, the hydrazine fuel, and the breakdown products of the latter on terrestrial microorganisms have been studied. The results indicate that the gaseous exhaust products would probably not be hazardous to microorganisms, but that liquid hydrazine would be lethal.

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